Original Article

Reversed phase-high performance liquid chromatographic method for simultaneous estimation of tolperisone hydrochloride and etodolac in a combined fixed dose oral formulations

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Abstract

A reversed-phase liquid chromatographic (RP-HPLC) method was developed for the simultaneous determination of tolperisone hydrochloride (TOLP) and etodolac (ETD) in a combined fixed dose oral formulation. The analysis was carried out using a phenomenax C-18, pre-packed column. A mobile phase containing a phosphate buffer (pH 5.5) : Methanol : Acetonitrile : Tri-ethylamine (40 : 40 : 20 : 1.5), with the pH adjusted to orthophosphoric acid, was pumped at a flow rate of 1.0 ml min^{1} with a UV-detector and PDA detection at 257 nm. Retention time was 3.91 minutes and 6.89 minutes for TOLP and ETD, respectively. The method was validated for linearity, accuracy, precision, sensitivity, and specificity. The method showed good linearity in the range of 3 – 21 μg ml for TOLP μg / ml and 8 – 56 μg / ml for ETD. The detection limit of the proposed method was 0.16 μg / ml and 0.58 μg / ml for TOLP and ETD, respectively. The quantification limit of the proposed method was 0.51 μg / ml and 1.7 μg / ml for TOLP and ETD, respectively. The % recovery was within the range of 99.42 – 101.15 for TOLP and 98.63 – 100.94 for ETD. The percentage RSD for precision of the method was found to be less than 2%. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The developed method could be applied for routine analysis of TOLP and ETD in tablet dosage form.

Key words: Etodolac, high performance liquid chromatography, tolperisone, validation

INTRODUCTION

Tolperisone HCL (TOLP), (R,S)-2-methyl-1-(4-methylphenyl)-3-piperidine-1-yl propan-1-one monohydrochloride is a centrally acting Spasmolytic agent used in the treatment of acute and chronic muscle spasms, various neurological diseases, electroconvulsive therapy, and orthopedic manipulation; Figure 1. TOLP is the subject of a monograph in the Japanese Pharmacopeia.[1,2] ETD, (R, S)-2-[1, 8-Diethyl-4, 9-dihidro-3H-pyrano (3, 4-b) indol-1-yl] acetic acid is a cox inhibitor, used as an analgesic to reduce pain in arthritis or acute injuries and to relieve moderate pain; Figure 2. ETD is the subject of the United States Pharmacopeia and British Pharmacopeia.[3-5] A combination of TOLP, 150 mg and ETD 400 mg has recently been approved for the treatment of acute muscle spasm. Literature reveals that potentiometric,[2] spectrophotometric,[6,7] and chromatographic methods[8-15] have been reported for their individual analysis, along with other combinations in pharmaceutical formulation and biological fluids. However, no method has been reported for their simultaneous determination in their combined fixed dose tablet formulation, to the best of our knowledge. The aim of the present study is to develop a feasible, rapid, sensitive, and specific HPLC method for the analysis of the investigated drugs.

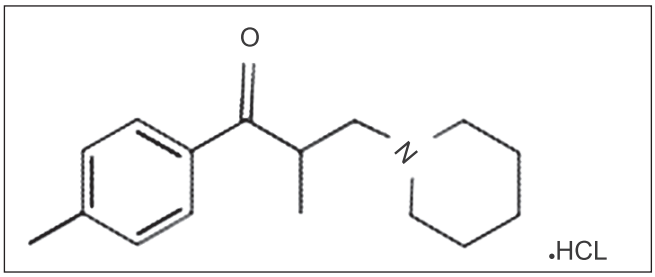

Figure 1: Structure of tolperisone hydrochloride

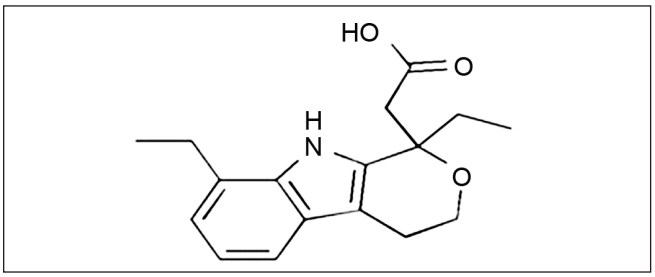

Figure 2: Structure of etodolac

MATERIALS AND METHODS

Chemical

The TOLP and ETD standard was obtained from Lupin Pharmaceuticals Ltd., Mumbai. The tablet formulation was obtained as a gift sample from Zydus Cadila Health Care Ltd. All the chemicals used were of Analytical Reagent grade and the solvents were of HPLC grade. HPLC grade water, methanol, acetonitrile, orthophosphoric acid, and tri-ethylamine (TEA) were purchased from S.D Fine Chemicals, Mumbai, India.

Apparatus

Separation was performed with a Shimadzu LC_10_ AT, equipped with a Rheodyne injector valve with a 20.0 µl loop and a UV / VIS detector, and the PDA (SPD_M_10_A VP) detector operated at 257 nm. The Class-VP software was applied for data collection and processing. A Chemline digital pH-meter was used for pH measurements.

Chromatographic conditions

A Phenomenex C18 column (150 mm x 4.6 mm, 5 µ) was used in this study. The mobile phase was a phosphate buffer (KH_2PO_4) of pH 5.5 : Methanol : Acetonitrile : Tri-ethylamine (40 : 40 : 20 : 1.5) adjusted to pH with orthophosphoric acid. The flow rate was 1.0 mL / minute and UV detection was performed at 257 nm by UV detector at 257 nm. The mobile phase was shaken on an ultrasonic bath for 30 minutes. The resulting transparent mobile phase was filtered through a 0.45-µm membrane filter (Millipore, Ireland).

Preparation of standard stock solutions

Stock solutions containing 150 µg / ml of TOLP and 400 µg / ml ETD were prepared in methanol and were used as working solutions. The solutions were kept in tight closed containers and were found to be stable for at least one week, when kept in the refrigerator.

Study of experimental parameters

Different experimental parameters including, mobile phase composition, detection wavelength, and flow rate were intensively studied, in order to specify the optimum conditions for the assay procedure. The variables were optimized by changing each, in turn, while keeping all others constant.

Construction of the calibration curve

Aliquots of the standard solutions covering the final working concentration range of 3.0 – 21.0 µg / ml for TOLP and 8 – 56 µg / ml ETD were transferred into a series of 10 ml volumetric flasks and diluted with the de-gassed mobile phase up to the mark. Aliquots of 20 µl were injected (n = 6) and eluted with the mobile phase under the reported chromatographic conditions. The calibration curves were constructed by plotting the peak area against the final concentration of the drug (µg / ml). Alternatively, the corresponding regression equations were derived.

Preparation of tolperisone hydrochloride and etodolac test solution

Twenty tablets were weighed accurately, taking an equivalent of 15 mg of TOLP : 40 mg of ETD tablet powder, and transferred to a 100 ml volumetric flask. This was sonicated with 60 ml methanol for 20 minutes and the volume was made up to 100 ml with the same solvent. This solution was filtered through a milipore filter. Four milliliters of this solution was transfered to a 100 ml volumetric flask and the volume was made up using the mobile phase.

Validation

The method was validated for assay of TOLP and ETD in accordance with ICH guidelines.[16]

Linearity

In order to check the linearity for the developed method, solutions of six different concentrations ranging from 3.0 – 21.0 µg / ml were prepared for TOLP and 8 – 56 µg / ml for ETD, respectively. The

chromatograms were recorded and the peak areas are given in Table 1. A linear relationship between areas versus concentrations was observed in the above-mentioned linearity range. This range was selected as the linear range for the development of the analytical method, for the estimation of TOLP and ETD.

Sensitivity

The sensitivity of the measurement of TOLP and ETD using the proposed method was estimated as the limit of quantification (LOQ) and the lowest concentration detected under these chromatographic conditions as the limit of detection (LOD). The LOD and LOQ were calculated by using the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S, where σ was the standard deviation of the peak areas of the drug (n = 6), and S was the slope of the corresponding calibration plot. The limits of detection and quantification for TOLP were 0.16 μg / ml and 0.51 μg / ml, respectively, and those for ETD were 0.58 μg / ml and 1.7 μg / ml, respectively.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values were within the limits, and are shown in Table 2. The statistical evaluation of the proposed method revealed its good linearity, reproducibility, and its validation of different parameters and led us to the conclusion that it could be used for the rapid and reliable determination of TOLP and ETD in tablet formulation. The results are furnished in Table 2.

Precision

Precision was measured by the analysis of sample solutions three times at three different concentrations. Solutions containing 3, 6, and 9 μg / ml of TOLP and 8, 16, and 24 μg / ml of ETD were subjected to the proposed HPLC analysis, to check the intraday and interday variations of the method. The results are furnished in Tables 3 and 4.

Accuracy

The accuracy of the method was determined by the analysis of standard additions at three levels, that is, multiple-level recovery studies. The reference standard, at three different concentrations (50, 100, and 150%), was added to a fixed amount of the preanalyzed sample and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in Tables 5 and 6.

Solution stability

The stability of TOLP and ETD standard and sample solutions was determined by storing the solutions at an ambient temperature (20 ± 10°C). The solutions were checked in triplicate after three successive days of storage and the data were compared with the freshly prepared samples. In each case, it could be noticed that the solutions were stable for 48 hours, as during this time the results did not decrease below

Table 1: Linearity data for tolperisone hydrochloride and etodolac

TOLP conc. (μg / ml)	Mean peak area of TOLP	ETD conc. (μg / ml)	Mean peak area of ETD
3.0	0223086	8.0	0169370
6.0	0450577	16.0	0314486
9.0	0669168	24.0	0498741
12.0	0900907	32.0	0644814
15.0	1129447	40.0	0813969
18.0	1343692	48.0	0966289
21.0	1583919	56.0	1123491

TOLP - Tolperisone hydrochloride; ETD - Etodolac

Table 2: System suitability parameters for tolperisone hydrochloride and etodolac

Parameter (*n = 5)	TOLP	ETD
Retention time (min)	3.91	6.89
Theoretical plates	18776	13428
Asymmetry	1.02	1.03
Capacity Factor	1.44	3.30
RSD (%)	0.67	1.06
Resolution	> 2	> 2

* Five replicates, TOLP - Tolperisone hydrochloride; ETD - Etodolac

Table 3: Results of the intraday precision

Tolperisone hydrochloride			Etodolac		
Conc. (μg / ml)	Peak area Mean S.D (n = 3)	RSD (%)	Conc. (μg / ml)	Peak area Mean ± S.D (n = 3)	RSD (%)
3	223919.7 ± 1704.725	0.76	8	168148 ± 1020.085	0.61
6	453040.3 ± 1973.407	0.44	16	315130.7 ± 2619.194	0.83
9	665532 ± 3348.36	0.51	24	500659.7 ± 4800.727	0.96

Table 4: Results of the interday precision

Tolperisone hydrochloride			Etodolac		
Conc. (μg / ml)	Peak area Mean ± S.D (n = 3)	RSD (%)	Conc. (μg / ml)	Peak area Mean ± S.D (n = 3)	RSD (%)
3	220336.7 ± 2055.112	0.93	8	168174.3 ± 2315.809	0.61
6	451989.0 ± 3072.652	0.68	16	315993.3 ± 3970.710	0.83
9	662058.3 ± 9727.580	1.47	24	495954.0 ± 7819.331	0.96

Table 5: Results of the recovery study of tolperisone hydrochloride

Amt of TOLP in sample (µg)	Amt. of Std. TOLP added (µg)	Total amt. of TOLP (µg)	Total amt. of TOLP found (µg) Mean ± S.D.	Total amt recovered (µg)	% Recovery (n = 3)
6.0	0	6.0	6.04 ± 791.34	-	-
6.0	3.0	09.0	9.07 ± 273.42	3.03	100.88
6.0	6.0	12.0	12.01 ± 543.58	5.96	99.42
6.0	9.0	15.0	15.16 ± 623.26	9.11	101.15

TOLP - Tolperisone hydrochloride

Table 6: Results of the recovery study of etodolac

Amt of ETD in sample (µg)	Amt. of Std. ETD added (µg)	Total amt. of ETD (µg)	Total amt. of ETD found (µg) Mean ± S.D.	Total amt. recovered (µg)	% recovery (n = 3)
16.0	0	16.0	15.93 ± 873.73	-	-
16.0	08.0	24.0	23.87 ± 743.36	7.94	99.28
16.0	16.0	32.0	32.08 ± 435.26	16.15	100.94
16.0	24.0	40.0	39.60 ± 865.63	23.67	98.63

ETD - Etodolac

Table 7: Results of the analysis of the test preparation

Formulation	(Mean ± % R.S.D.)	
	TOLP	ETD
%conc. estimated*	100.66 ± 0.46	99.56 ± 0.74

* Average of six determinations; R.S.D.: Relative standard deviation
TOLP - Tolperisone hydrochloride; ETD - Etodolac

98%. This showed that TOLP and ETD were stable in standard and sample solutions for at least two days, at ambient temperature.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate (± 0.1), temperature (± 5), and pH (± 0.2) of the mobile phase. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop an accurate and stable assay method with the pure drugs TOLP and ETD, in a tablet formulation. A Phenomenax C18 (150 mm x 4.6 mm, 5 µ) column in isocratic mode was used, with a mobile phase phosphate buffer (KH_2PO_4, pH 5.5) : Methanol : Acetonitrile : Tri-ethylamine (40 : 40 : 20 : 1.5); pH of the buffer adjusted with orthophosphoric acid. The flow rate was 1 mL / minute and identical components were measured, with detection at 257 nm. Linearity was assessed by plotting concentration versus area, which is shown in Table 1, and it is linear in the range of 3.0 – 21.0 µg / ml for TOLP and 8.0 – 56.0 µg / ml for ETD, with correlation coefficients of 0.9998 and 0.9995, respectively, with a good linearity response, greater than 0.999. The % recovery was found to be within limits of the acceptance criteria with a recovery range of 99.42 – 101.15% for TOLP and 99.28 – 100.94% for ETD. The %RSD for intraday and Interday precision was less than 2% for TOLP and ETD. The detection limit of the proposed method was 0.16 µg / ml and 0.58 µg / ml, and the quantification limit was 0.51 µg / ml and 1.7 µg / ml for TOLP and ETD, respectively. A typical overlain chromatogram of the standard solution is shown in Figure 3, chromatogram of the standard solution of TOLP and ETD at the test level is shown in Figure 4, and a chromatogram of the test solution is shown in Figure 5. The assay procedures were repeated six times and the results were found to give 100.66% of TOLP and 99.56 % of ETD as shown in Table 7.

CONCLUSIONS

The proposed study describes a new and simple RP-HPLC method for the estimation of TOLP and ETD in tablet formulation. The method has been validated and found to be simple, rapid, sensitive, accurate, and precise. Therefore, the proposed method can be used for quantification of TOLP and ETD in solid oral formulations as well as routine analysis, in quality control.

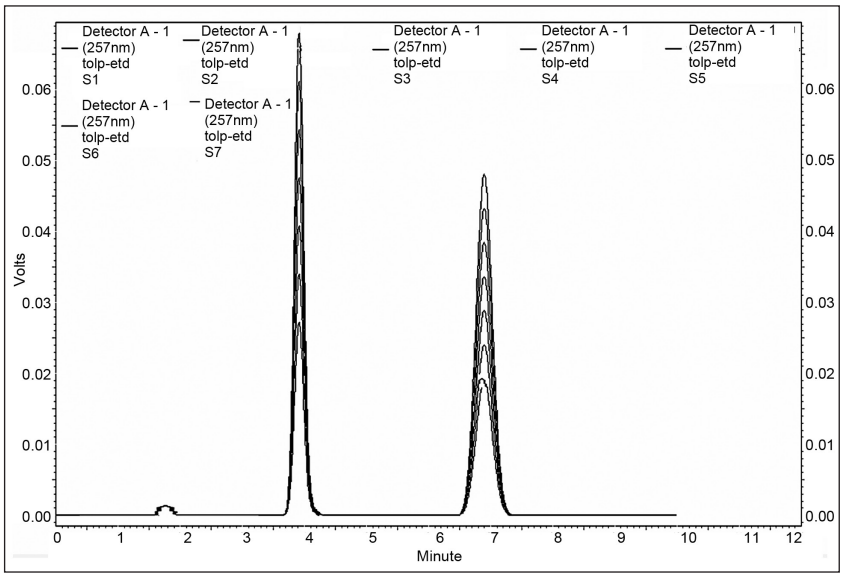

Figure 3: Overlain chromatogram of tolperisone hydrochloride and etodolac

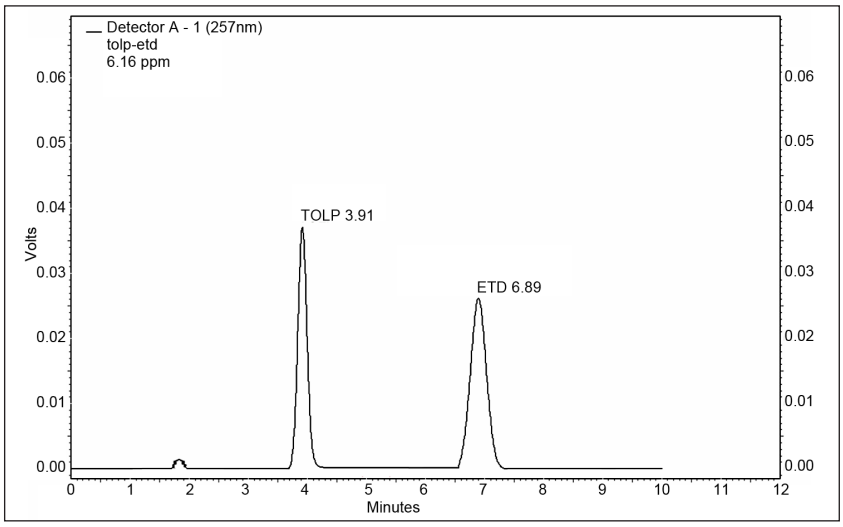

Figure 4: Chromatogram of the standard preparation of tolperisone hydrochloride and etodolac

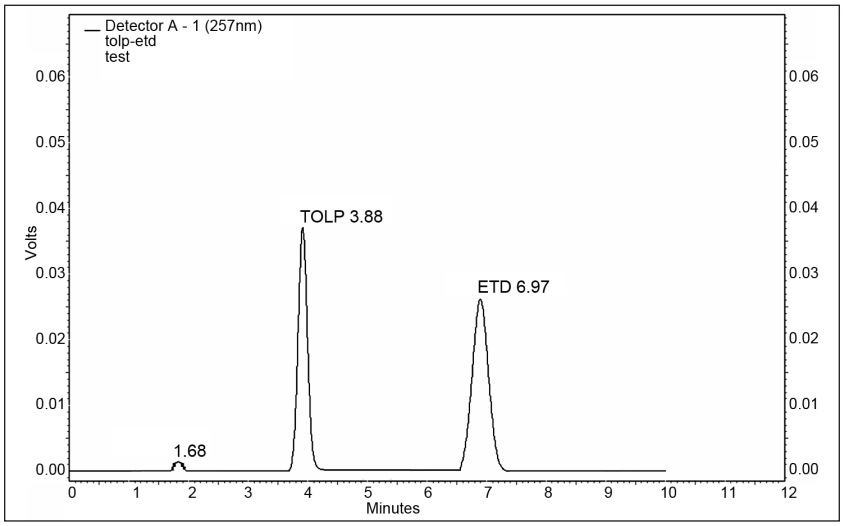

Figure 5: Chromatogram of the test preparation of tolperisone hydrochloride and etodolac

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